

Immobility-reducing Effects of Ketamine during the Forced Swim Test on 5-HT_{1A} Receptor Activity in the Medial Prefrontal Cortex in an Intractable Depression Model

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Ketamine has been clinically proven to ameliorate depression, including treatment-resistant depression. The detailed mechanism of action of ketamine in treatment-resistant depression remains unclear. We examined the effects of ketamine on the immobility times of adrenocorticotrophic hormone (ACTH)-treated rats during the forced swim test, and we explored the mechanism by which ketamine acts in this model. We investigated the neuroanatomical site of action by microinjecting ketamine into the medial prefrontal cortex of rats. A significant reduction of the rats' immobility during the forced swim test was observed after the intraperitoneal injection of ketamine in both saline- and ACTH-treated rats. The microinjection of ketamine into the medial prefrontal cortex also decreased immobility during the forced swim test in both saline- and ACTH-treated rats. The immobility-decreasing effect of intraperitoneally injected ketamine was blocked by administering WAY100635, a 5-HT_{1A} receptor antagonist, into the medial prefrontal cortex. These findings contribute to the evidence that ketamine can be useful against treatment-resistant depressive conditions. The immobility-reducing effects of ketamine might be mediated by 5-HT_{1A} receptor activity in the medial prefrontal cortex.

Key words: ketamine, adrenocorticotrophic hormone, forced swim test, medial prefrontal cortex, 5-HT_{1A} receptor

Neuroendocrinological studies of patients with depression have focused on the hypothalamic-pituitary-adrenal (HPA) axis, and depressed patients have been reported to have dysfunctional HPA axes [1]. We and others have reported that the chronic administration of adrenocorticotrophic hormone (ACTH) for 14 days to rats counteracted the effects of the tricyclic antidepressants imipramine and desipramine and decreased the amount of time the rats spent immobile in the forced swim test, a behavioral test that is widely used as a predictor of antidepressive activity [2, 3]. The chronic

treatment of rats with ACTH might therefore represent an effective animal model of antidepressant treatment-resistant depression.

Ketamine is a non-competitive antagonist of the phencyclidine site of the N-methyl-D-aspartate (NMDA) receptor. Clinically, it is well known that ketamine exerts rapid and sustained antidepressive effects in depressed patients and treatment-resistant depressive patients [4-6]. In 2019, the U.S. Food and Drug Administration approved esketamine as a treatment for treatment-resistant depression. It was reported that in rodents, ketamine significantly decreased immobility in

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the forced swim test [7,8], and that ketamine significantly decreased the amount of time ACTH-treated rats spent immobile during the forced swim test [3]. These results suggested that ketamine could be effective against antidepressant treatment-resistant depression.

The serotonin (5-HT)_{1A} receptor has been implicated in the pathophysiology of major depressive disorder in both animal models and humans [9,10]. We observed that the 5-HT_{1A} receptor full agonist (\pm)-8-hydroxy-2-(di-n-propylamino)tetralin significantly reduced the amount of time saline- and ACTH-treated rats spent immobile during the forced swim test [11]. Thus, the 5-HT_{1A} receptor might play an important role in depression, particularly in depression that is resistant to tricyclic antidepressant treatment. The antidepressive effects of ketamine might also be mediated by post-synaptic 5-HT_{1A} receptor activity in mice [12]. In mice that underwent the novelty-suppressed feeding test, the antidepressive effects of ketamine were attenuated by WAY100635, a 5-HT_{1A} receptor antagonist, but not ritanserin, a 5-HT_{2A/2C} receptor antagonist [13]. These findings suggested that ketamine exerts its effects by indirectly activating postsynaptic 5-HT_{1A} receptors. Ketamine might therefore be effective in animal models of depressive conditions that are resistant to tricyclic antidepressant treatment, and these effects might be mediated by post-synaptic 5-HT_{1A} receptor activity.

The role of the medial prefrontal cortex (MPC) in depression has been extensively studied. Conditioned fear stress selectively increased 5-HT metabolism in the MPC [14]. Ketamine increased the release of 5-HT from the MPC, probably by activating neurons in the dorsal raphe nucleus [15,16]. Following its microinjection into the MPC of rodents, ketamine was observed to exert antidepressive effects in the forced swim test, and these effects were attenuated by depleting 5-HT using p-chlorophenylalanine, an inhibitor of 5-HT synthesis [17]. These findings suggested that the MPC serotonergic system is deeply involved in the psychopathology of depression and anxiety.

We conducted the present study to investigate whether ketamine treatment would reduce the amount of time ACTH-treated rats spent immobile during the forced swim test. To explore the role of the 5-HT_{1A} receptor in the antidepressant-like effects of ketamine, we also examined whether the effects of ketamine are mediated via 5-HT_{1A} receptors in the MPC.

Materials and Methods

Animals. Wistar rats (Charles River, Yokohama, Japan) with initial weights of 210-230 g were used. The rats were housed 2 per cage in an air-conditioned room (23 \pm 1°C; approximate humidity level: 60%) under a constant light-dark cycle (lights on: 7:00 a.m. to 7:00 p.m.) and were fed standard laboratory food and tap water. All experiments were conducted according to the guidelines for animal experimentation at Okayama University Medical School. All efforts were made to minimize the number of animals used and the degree of suffering they experienced.

Drug administration. The following drugs were used: ketamine (Ketalar[®]; Daiichi-Sankyo, Tokyo), WAY100635 (Sigma-Aldrich, St. Louis, MO, USA), and ACTH-(1-24)-zinc (Cortrosyn Z; Daiichi-Sankyo). The rats were administered ketamine intraperitoneally at a dose of 2 ml/kg body weight. ACTH was injected subcutaneously once daily (at 9:00-10:00 a.m.) for 14 days at a dose of 100 μ g/rat (injection volume: 0.2 ml/rat). The control rats received an equivalent volume of vehicle (saline, 0.2 ml/rat, subcutaneously injected) for the same period of time. The amount of time the control rats spent immobile was assessed 60 min after the administration of a single dose of ketamine (10-20 mg/kg, i.p.) to rats that had been treated with ACTH for 14 days. The last injection of ACTH was given immediately after the pre-swim test. Ketamine was administered the next day, without concurrent ACTH treatment. The amount of time the rats spent immobile was assessed 60 min after the administration of ketamine as described below.

Microinjections. Rats were anesthetized by a single intraperitoneal administration of sodium pentobarbital (50 mg/kg) and were fixed to a brain stereotactic apparatus (Narishige, Tokyo). For the injection of ketamine into the MPC, the rat's brain was implanted with bilateral guide cannulas (AG-8; Eicom, Kyoto, Japan), and the tips were positioned in the MPC (anteroposterior: +3.2 mm in front of the bregma suture, midline: \pm 0.6 mm, dorsoventral: -3.2 mm, angle: 20°). The cannulas were held in place with dental cement. A dummy cannula was inserted into the guide cannula to prevent clogging.

The microinjection of ketamine or WAY100635 was performed on day 2 after surgery. The injection cannula (AMI-8; Eicom Co., Kyoto, Japan) was connected via

Teflon tubing to a Hamilton microsyringe driven by a microinfusion pump (CMA/100 syringe pump; Carnegie Medicine, Stockholm, Sweden). The injections were performed for 1 min at a rate of 1 μ l/min. The injection cannulas were left in position for an additional 1 min before being withdrawn. After the behavioral test, Evans blue dye was infused and then coronal sections were prepared to confirm the locations of the cannula tips. The ketamine and WAY100635 were diluted with Ringer's solution prior to being used for the MPC microinjections.

Assessment of immobility. For the assessment of immobility, individual rats were placed in plastic cylinders (height 37 cm, dia. 15.5 cm), containing 20 cm of water at 25°C, as described by Porsolt *et al.* [18]. Two swimming sessions were conducted in the initial 13-min pretest, and a 6-min test was performed 24 h later. The total amount of time each rat spent immobile during the 6-min test period was recorded by a TARGET series/7M analysis program (Neuroscience Inc., Tokyo).

On the 15th day of treatment, immobility was assessed 60 min after the administration of a single dose of ketamine (10-20 mg/kg, i.p.) without ACTH. In the microinjection experiments, immobility was assessed 15 min after the microinjection of ketamine (1-15 μ g/side) into the MPC without ACTH on the 15th day of treatment. In the experiment involving the microinjection of WAY100635, WAY100635 (1 nmol/side) was administered 20 min before the assessment of mobility.

Locomotor activity. The rats' locomotor activity was monitored for 6 min with the use of automated activity monitoring chambers (Neuroscience, Inc.). The plastic chambers measured 23 cm (width) by 40 cm (length) by 18 cm (height).

Statistical analyses. All data are expressed as mean \pm standard error of the mean (SEM) values. The immobility time and locomotor activity were analyzed by a one-way analysis of variance (ANOVA). The group means were compared using Dunnett's test (Figs. 1, 2; Table 1) and Tukey's test (Fig. 3) for multiple comparisons. For the evaluation of the influence of the ketamine injection into the MPC on the rats' locomotor activity, comparisons between the 2 groups were performed using the unpaired two-tailed Student's *t*-test. *P*-values of <0.05 were considered significant.

Results

Effects of ketamine on immobility in saline- and ACTH-treated rats.

We examined the amount of time that the saline- and ACTH-treated rats spent immobile during the forced swim test after they were treated with ketamine (10-20 mg/kg, i.p.). Ketamine significantly reduced the amount of time spent immobile during the forced swim test in both the saline- and ACTH-treated rats (control: $F(3, 20) = 7.31$, $p < 0.01$; ACTH: $F(3, 20) = 3.11$, $p < 0.05$) (Fig. 1).

Effects of the infusion of ketamine into the MPC on the amount of time saline- and ACTH-treated rats

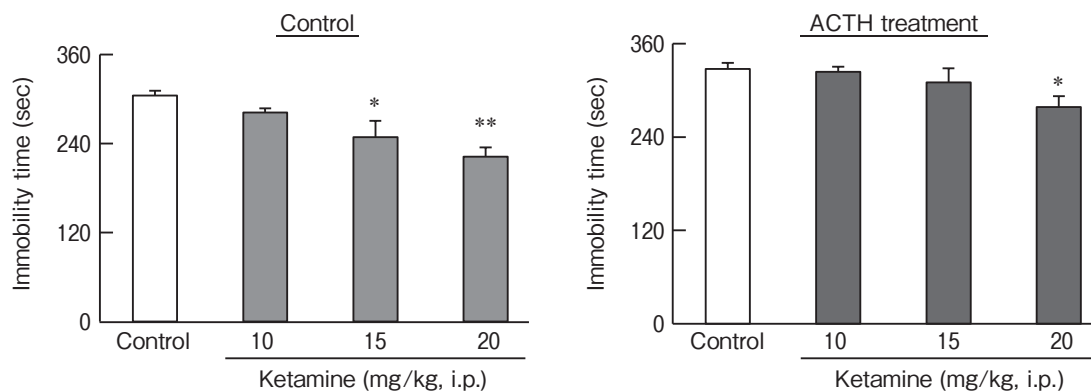


Fig. 1 The effects of ketamine on the amount of time saline- or ACTH-treated rats spent immobile during the forced swim test. Immobility was assessed 60 min after the administration of ketamine (10-20 mg/kg, i.p.) in saline- and ACTH-treated rats. We repeatedly administered ACTH (100 μ g/day, s.c., 14 days) to rats for 14 days. On the 15th day, ketamine was administered without ACTH. Each column represents the mean \pm SEM ($n = 6$ for each group). Data were analyzed with a one-way ANOVA, and group means were compared using Dunnett's test for multiple comparisons. * $P < 0.05$ vs. the control group. ** $P < 0.01$ vs. the control group.

spent immobile. We examined the effects of ketamine (1–10 $\mu\text{g}/\text{side}$) injected into the MPC on the amount of time saline- and ACTH-treated rats spent immobile during the forced swim test (Fig. 2). The infusion of 10 $\mu\text{g}/\text{side}$ ketamine into the MPC decreased the amount of time the control rats spent immobile during the forced swim test ($F(3, 15) = 8.77$, $p < 0.01$). Similarly, the infusion of 15 $\mu\text{g}/\text{side}$ ketamine into the MPC significantly decreased the amount of time the ACTH-treated rats spent immobile during the forced swim test ($F(2, 11) = 4.48$, $p < 0.05$) (Fig. 2).

Effects of ketamine on locomotor activity in saline- and ACTH-treated rats. Ketamine (20 mg/kg, i.p.) significantly decreased the locomotor activity of the saline- and ACTH-treated rats (saline: $F(3, 20) = 0.001$, $p < 0.01$; ACTH: $F(3, 20) = 3.13$, $p < 0.05$) (Table 1). In contrast, the injection of 10 or 15 $\mu\text{g}/\text{side}$ ketamine into the MPC did not affect locomotor activity in the saline- or ACTH-treated rats (Table 2).

Effects of the microinjection of WAY100635 into the MPC on the immobility-decreasing effects of ketamine in the forced swim test. The effects of the microinjection of WAY100635 into the MPC on the ketamine-induced reduction of time the rats spent immobile during the forced swim test are illustrated in Fig. 3. The reduction in immobility induced by ketamine (20 mg/kg, i.p.) was prevented by microinjecting WAY100635 (1 nmol/side) into the MPC ($F(3, 39) = 3.71$, $p < 0.05$).

Discussion

Our findings clarify the effects of an intraperitoneal injection of ketamine and a microinjection of ketamine into the MPC on the amount of time ACTH-treated rats spent immobile during the forced swim test. The observed effects of ketamine (i.p.) agree with the results obtained for normal and ACTH-treated rats in previous studies [3, 12].

In a study using mice, the microinjection of ketamine into the MPC decreased immobility [16, 17], and

Table 1 The effects of ketamine on locomotor activity in saline- and ACTH-treated rats

Dose (mg/kg)	Locomotor activity (counts)	
	Saline	ACTH
–	221.3 \pm 6.8	222.5 \pm 10.0
10	205.1 \pm 19.1	202.9 \pm 13.7
15	184.1 \pm 8.3	182.9 \pm 14.1
20	134.4 \pm 18.3*	181.9 \pm 18.4

ACTH (100 g/rat, s.c.) was administered to the rats once daily for 14 days. Locomotor activity was measured on the day after the final dose of ACTH was administered, 60 min after the ketamine injection. Data are mean \pm SEM values ($n = 5$ – 6 rats). The data were analyzed with a one-way ANOVA, and group means were compared using Dunnett's test for multiple comparisons. * $P < 0.05$ vs. the saline-treated group.

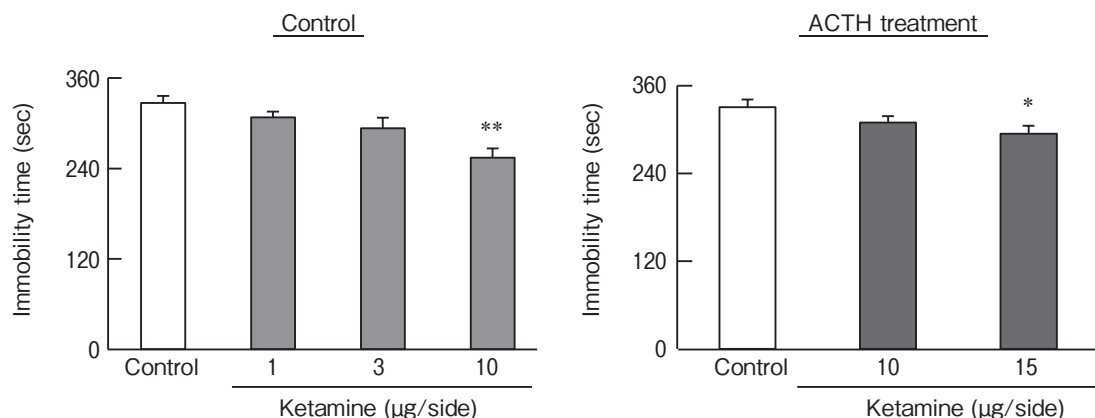


Fig. 2 The effects of the microinjection of ketamine into the MPC for 14 days on the amount of time saline- or ACTH-treated rats spent immobile during the forced swim test. We examined the effects of injecting a single dose of ketamine (1–15 $\mu\text{g}/\text{side}$) into the MPC on the amount of time rats spent immobile during the forced swim test. Immobility was assessed 15 min after the administration of ketamine. We repeatedly administered ACTH (100 $\mu\text{g}/\text{day}$, s.c., 14 days) to rats for 14 days. On the 15th day, ketamine was administered without ACTH. Each column represents the mean \pm SEM ($n = 4$ – 6 , each group). Data were analyzed with a one-way ANOVA, and group means were compared using Dunnett's test for multiple comparisons. * $P < 0.05$, ** $P < 0.01$ vs. the control group.

Table 2 Influence of the infusion of ketamine into the medial prefrontal cortex (MPC) on locomotor activity in saline- and ACTH-treated rats

a) Saline

Treatment	Locomotor activity (counts)
Saline	165.0 ± 12.2
Ketamine (10 µg/side)	181.2 ± 15.0

b) ACTH

Treatment	Locomotor activity (counts)
ACTH	201.5 ± 9.3
ACTH + ketamine (15 µg/side)	189.0 ± 12.3

ACTH (100 g/rat, s.c.) was administered to the rats once daily for 14 days. Locomotor activity was measured on the day after the final dose of ACTH was administered, 15 min after the infusion of ketamine into the MPC. Data are expressed as the mean ± SEM values ($n=5-6$ rats). The data were analyzed with the unpaired two-tailed Student's *t*-test.

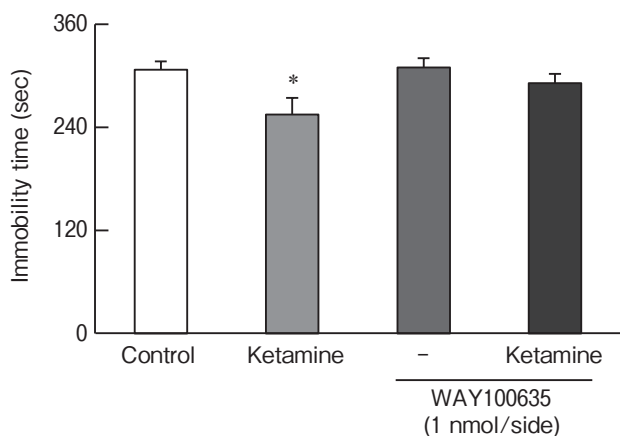


Fig. 3 The effects of microinjecting WAY100635 into the MPC on the immobility-reducing effects of ketamine in rats during the forced swim test. Immobility was assessed 60 min after the administration of ketamine (20 mg/kg, i.p.). WAY100635 (1 nmol/side) was injected into the MPC 20 min before the examination. Each column represents the mean ± SEM ($n=9-12$, each group). Data were analyzed with a one-way ANOVA, and group means were compared using Tukey's test for multiple comparisons. * $P < 0.05$ vs. the control group.

in the present investigation using rats, the infusion of ketamine at a dose of 10 µg/side into the MPC significantly reduced the amount of time the control rats spent immobile during the forced swim test. In the ACTH-treated rats, the infusion of ketamine at 15 µg/side into the MPC significantly reduced the amount of time they

spent immobile during the forced swim test. Taken together, the previous and present results suggest that ketamine may improve the antidepressant treatment-resistant depression.

To determine whether the observed changes in immobility were associated with reductions in locomotor activity, we examined the effects of ketamine on locomotor activity in saline- and ACTH-treated rats. Only the highest dose of ketamine (20 mg/kg, i.p.) decreased the locomotor activity of both the saline- and ACTH-treated rats. It is therefore unlikely that the tendency for ketamine to reduce immobility in the forced swim test was related to the drug's effect on locomotor activity.

In mice, the antidepressive effects of ketamine appear to be mediated by 5-HT_{1A} receptor activity in the MPC [12]. We reported the effects of 8-OH-DPAT, a 5-HT_{1A} receptor agonist, on the immobility exhibited by saline- or ACTH-treated rats during the forced swim test [11], and we observed that 8-OH-DPAT potently reduced the amount of time the ACTH-treated rats spent immobile during the forced swim test, in a dose-dependent manner. This suggests that the 5-HT_{1A} receptor plays an important role in depression, particularly in depression that is resistant to tricyclic antidepressant treatment. We also observed that the microinjection of WAY100635 (a 5-HT_{1A} receptor antagonist) into the MPC blocked the immobility-decreasing effects of ketamine. The present results thus indicate that the stimulation of 5-HT_{1A} receptors in the MPC might play an important role in the antidepressive effects of ketamine.

It was reported that the sustained antidepressant-like effects of ketamine increase the release of 5-HT in the MPC through local mechanisms, such as inhibition of the 5-HT transporter, or through the activation of the dorsal raphe nucleus neurons by MPC projection [12]. A recent study concerning 5-HT_{1A} receptor function revealed that the antidepressant-like effects of ketamine are mediated by the post-synaptic 5-HT_{1A} receptor and the subsequent activation of phosphoinositide-3 kinase/Akt/mechanistic target of rapamycin complex-1 (PI3K/Akt/mTORC1) signaling in the MPC [19]. Ketamine is a non-competitive NMDA receptor antagonist. In the glutamatergic system, the metabotropic glutamate (mGlu) receptors are known to have modulatory roles in glutamatergic transmission, and their roles in depression have been investigated [20]. Importantly,

an mGlu2/3 receptor antagonist had rapid and long-lasting ketamine-like antidepressant effects on treatment-resistant depression in rodents [19,21-23]. Further studies are in progress to clarify the detailed mechanisms underlying the effects of ketamine in ACTH-treated rats.

In conclusion, the present results demonstrated that ketamine exerted antidepressant-like effects via the medial prefrontal cortex in both control and ACTH-treated rats. Ketamine might have important effects on depression that are mediated through 5-HT_{1A} receptors in the medial prefrontal cortex.

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